IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Merril et al.
Appl. No.	:	10/659,711
Filed	:	September 11, 2003
For	:	ANTIBACTERIAL THERAPY WITH BACTERIOPHAGE GENOTYPICALLY MODIFIED BY GENETIC ENGINEERING TO DELAY INACTIVATION BY THE HOST DEFENSE SYSTEM
Examiner	:	Snyder, Stuart
Group Art Unit	:	1648

DECLARATION UNDER 37 CFR 1.132 OF CARL R. MERRIL, M.D.

I, Carl R. Merril, M.D., do hereby declare:

1. I am a named inventor of the above-identified application. A true and correct copy of my Curriculum Vitae has been previously submitted with my Declaration filed on August 15, 2006 in connection with the present application.

2. The invention described in the above-referenced application solves the problem in the prior art of the use of bacteriophage to fight infections caused by bacteria. We reasoned that one explanation for bacteriophage not always working was because the viruses were inactivated by the host innate immune system. To solve this problem, we developed a technology to produce bacteriophage that is genetically modified to delay inactivation by the host's innate immune system.

3. As described in our patent application, one of the ways to delay inactivation by the host defense system is to engineer a phage to express molecules that antagonize one or more of the host defense components. It was known that viruses that infect animals encode inhibitors of complement activation to evade host immune responses. See, e.g., Isaacs et al., Proc Natl Acad Sci USA 89: 628 (1992). Bacteriophage did not evolve to express immune response genes like poxviruses because they infect bacteria not animals.

4. As described in our patent application, complement components fix to bacteriophages, and these bacteriophages then adhere to certain white blood cells (such as macrophages) that express complement receptors. Numerous peptides have been synthesized that antagonize the functions of the various complement components. See, e.g., Lambris, J. D. et al, "Use of synthetic peptides in exploring and modifying complement reactivities" in Activators and Inhibitors of Complement, ed. R. Sim, Kluwer Academic Publishers, Boston, 1993. Lambris et al. (op.cit.) cite "a series of synthetic peptides spanning the convertase cleavage site in C3 (that are) found to inhibit complement activation by both the classical and alternative pathways." Among the peptides cited is a six amino acid peptide (LARSNL, residues 746-751 of C3) that "inhibits both pathways equally well." Thus, one example of how to delay inactivation by the host defense system is to engineer a phage to express molecules that antagonize one or more of the various complement components.

5. As described in our patent application, in one method of genetically engineering a phage capable of delaying inactivation by the host defense system, a fusion protein is obtained, wherein the peptide will be bound to the carboxyl end of the surface protein of interest. See, e.g., Sambrook, J., Fritsch, E., and Maniatis, T.: Molecular Cloning. A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. This construct is made by cloning the gene for the phage surface protein into a plasmid vector system, and then cloning the oligonucleotide for the peptide of interest into this carrying vector by in-frame fusion at the 3'-end of the gene for the surface protein. This fusion of the gene for the phage surface protein with an oligonucleotide for a host defense element, such as a complement-antagonizing peptide, would then be incorporated into the phage of interest by the in vivo generalized recombination system in the host bacteria for the phage of interest. Phage whose genomic sequence is already completely known, and phage whose genomic sequence is unknown or partially unknown can be used in the present invention.

6. Our patent application describes at Example 4, genetic engineering of phage to express molecules that antagonize the host defense system, thereby enabling the phage to delay inactivation by the host defense system. It includes as an example the use of the orfx gene, which encodes a carboxy-terminal tail protein of lambda coliphage, one for which it is known

that foreign nucleotide sequences can be introduced without there being disruption of the structure or function of the phage. Montag et al., J Bacteriol 171: 4378 (1989). The tail surface protein expressed by the orfx gene is made into a fusion protein with the host defense antagonizing peptide, by the plasmid vector method described in Example 4 of the application.

7. Our patent application describes at Example 5, that the genetically engineered phage delay inactivation by the host innate immune system, compared to wild-type phage and describes at Example 6, that the genetically engineered phage has a greater capacity than wild type phage to prevent lethal infections in mice.

8. The claims at issue in this application, Claims 20, 22 and 23, recite a method of producing a bacteriophage able to delay inactivation by an animal's innate immune system, comprising genetically engineering the bacteriophage by fusing a gene for a surface protein with an oligonucleotide for a host defense antagonizing peptide to create a fusion protein, such that said fusion protein is expressed on the surface coat of the bacteriophage.

9. The state of the art at the April 1994 time of filing the present application with regard to predictability of altering the phage surface to delay inactivation by an animal's innate immune system can be determined by several references previously submitted and of record in this application. Specifically, Lambris, J. D. et al, "Use of synthetic peptides in exploring and modifying complement reactivities" in Activators and Inhibitors of Complement, ed. R. Sim, Kluwer Academic Publishers, Boston, 1993, is a representative paper exemplifying the state of the art with regard to peptides that modify complement reactivities foreign to bacteriophages. Isaacs et al., Proc Natl Acad Sci USA 89: 628 (1992) shows that viruses that infect animals encode proteins that inhibit complement activation, thus inhibiting complement activation is compatible with the viability of a virus. Montag et al., J Bacteriol 171: 4378 (1989) is a representative paper exemplifying the state of the art with regard to the display of a foreign peptide on the surface of a phage while preserving infectivity. The conclusion to be drawn from these papers is that altering the phage surface to delay inactivation by an animal's innate immune system through specific genetic engineering techniques was enabled by the patent specification in view of the state of the art at the April 1994 time of filing.

10. In addition, Gupta et al., J. Mol. Biol. 334, 241-254 (2003) describes a phage designed to display peptides and proteins fused at the C terminus of the head protein gpD of phage lambda. Bair et al., Mol. Micro. 67[4], 719-728 (2008) describe a bacteriophage system having proteins displayed as fusions to the surface of the phage. Edgar et al., PNAS 103:13, 4841-4845 (2006) describe a genetically engineered phage that expresses the major capsid protein gp10A fused to a 15-aa biotinylated peptide that results in the display of the peptide on the major capsid protein. These references provide evidence that the method set forth in the pending claims, that is, genetically engineering a bacteriophage by fusing a gene for a surface protein with an oligonucleotide for a desired peptide to create a fusion protein, such that said fusion protein is expressed on the surface coat of the bacteriophage, is fully enabled by the disclosure set forth in the specification. (The references cited above are being submitted herewith in an Information Disclosure Statement).

11. Further, Sokoloff et al., Molecular Therapy, (2000) 2:2, 131-139, describe phage displaying peptides at the carboxy-terminus of the phage coat proteins that avoid inactivation by complement. (This reference is also submitted herewith in an Information Disclosure Statement). Thus, this reference supports our disclosure in the specification that through genetic engineering, the phage can be modified such that the fusion protein expressed on the surface coat of the phage is able to delay inactivation by an animal's innate immune system.

12. In summary, one of skill in the art would know how and where to insert the complement-antagonizing peptide to create a fusion protein expressed on the surface coat of the phage so as to delay inactivation by the animal's innate immune system. The specification provides enough information to permit one of skill in the art to recreate the phage using the claimed method, in view of the state of the art at the April 1994 time of filing. This position is supported by later-published articles that describe the genetic engineering of phage to create a fusion protein expressed on the surface of the coat of the phage that delays inactivation by the animal's innate immune system.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

Dated: 8 July 2008

By: Carl R. Merril MD
Carl R. Merril, M.D.

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